Figure S1 The maximum-likelihood tree of mammalian *TAS2R* genes.

All the mammalian *TAS2R* gene clusters identified in the platypus and echidna genome project (Zhou *et al.* 2021) were confirmed in this tree. Human *V1R68* and *V1R77* were used as outgroups. The nodes of each orthologous gene group are marked as open circles (supported by ≥ 95 % bootstrap values). The nodes connecting orthologous gene groups with ≥ 70 % bootstrap values are marked as asterisks. Species abbreviations are as follows; *Oan*: platypus; *Tac*: echidna; *Hsa*: human; *Mmu*: mouse; *Bta*: cattle; *Fca*: domestic cat; *Mdo*: opossum; *Sha*: Tasmanian devil; *Pci*: koala; *Gga*: chicken.
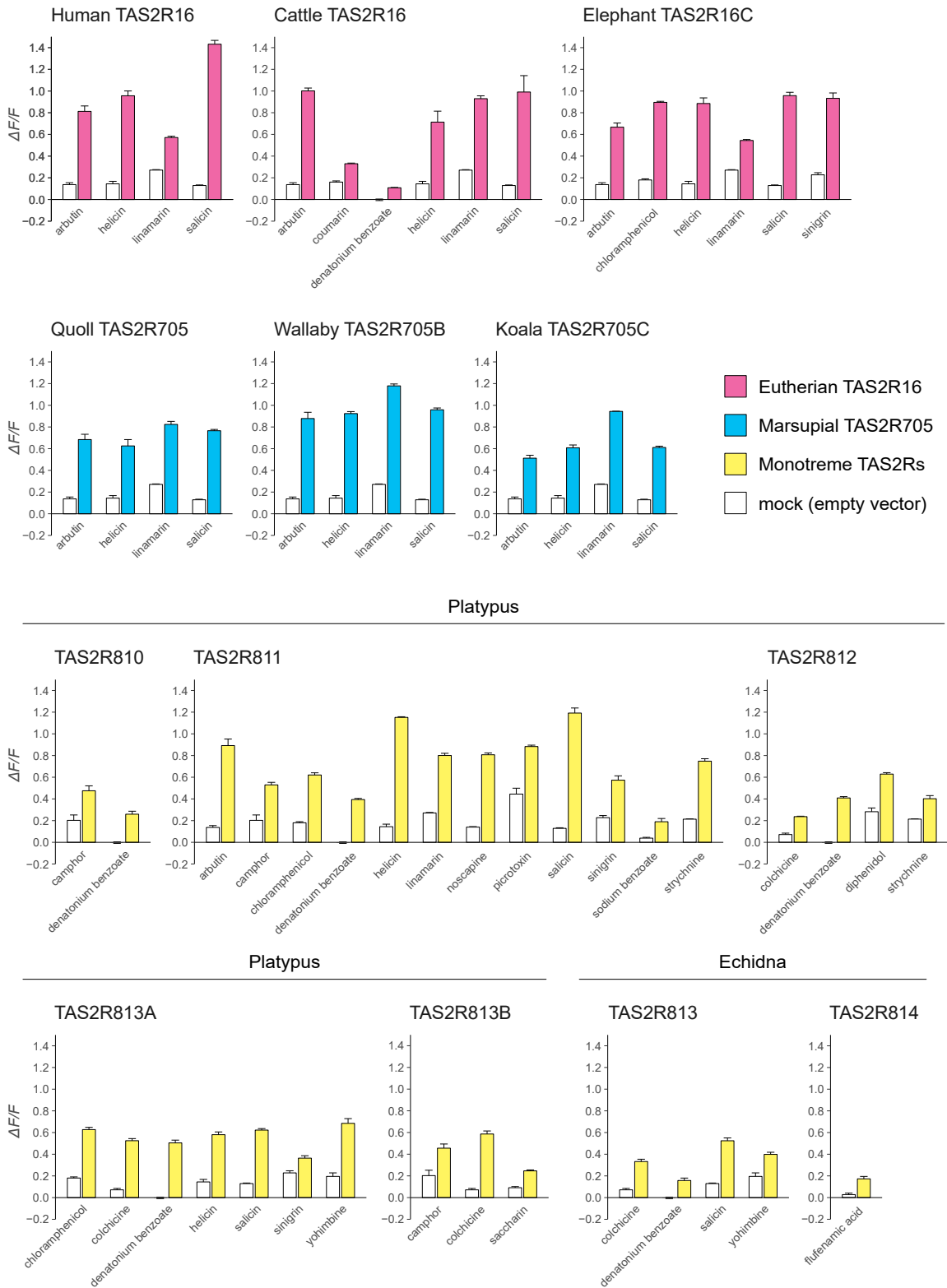
Figure S2 Responses of TAS2Rs to the identified agonists.

The ligand-receptor pairs that TAS2R-expressing cells showed significantly higher responses than mock-transfected cells (Dunnett's test, $p < 0.01$) are visualized (n = 3-5).

Table S1 Whole genome assemblies used in this study

Species	Assembly	Reference[1]
Homo sapiens	GRch38.p13	Schneider et al., 2017
Mus musculus	GRCm39	MGSC 2002
Bos taurus	ARS-UCD1.2	Rosen et al., 2020
Felis catus	Felis_catus_9.0	Buckley et al., 2020
Loxodonta africana	LoxAfr3.0	Lindblad-Toh et al., 2011
Echinops telfairi	ASM31398v2	Zoonomia Consortium 2020
Dasypus novemcinctus	dasNov3	Lindblad-Toh et al., 2011
Monodelphis domestica	MonDom5	Mikkelsen et al., 2007
Sarcophilus harrisii	Devil_ref v7.0	Murchison et al., 2012
Phascolarctos cinereus	phaCin_unsw_v4.1	Johnson et al., 2018
Ornithorhynchus anatinus	mOrnAna1.pri.v4	Zhou et al., 2021
Tachyglossus aculeatus	mTacAcu1.pri	Zhou et al., 2021
Gallus gallus	GRCg6a	ICGSC 2004

[1] MGSC: Mouse Genome Sequencing Consortium; ICGSC: International Chicken Genome Sequencing Consortium.

Table S2 Localization of TAS2Rs and their adjacent genes.

Species	Gene	Location	Strand
Homo sapiens	**TAS2R16**	NC_000007.14:122994759-122995634	-
	TAS2R62P	NC_000007.14:143437029-143437973	+
	TAS2R60	NC_000007.14:143443453-143444409	+
	TAS2R41	NC_000007.14:143477873-143478796	+
	AASS	NC_000007.14:122073544-122144269	-
	CADPS2	NC_000007.14:122318411-122886500	-
	SLC13A1	NC_000007.14:123113490-123201836	-
	GSTK1	NC_000007.14:143263441-143269115	+
	CLCN1	NC_000007.14:143316111-143352083	+
	ZYX	NC_000007.14:143381345-143391111	+
	EPHA1	NC_000007.14:143391129-143408856	-
	ARHGEF5	NC_000007.14:144355396-144380632	+
	NOBOX	NC_000007.14:144396805-144410422	-
Mus musculus	**Tas2r118**	NC_000072.7:23969160-23970059	-
	Tas2r143	NC_000072.7:42377172-42378053	+
	Tas2r134	NC_000068.8:51517523-51518419	+
	Tas2r135	NC_000072.7:42382490-42383428	+
	Tas2r126	NC_000072.7:42411469-42412395	+
	Aass	NC_000072.7:23072172-23132985	-
	Cadps2	NC_000072.7:23262772-23840662	-
	Slc13a1	NC_000072.7:24088282-24168118	-
	Gstk1	NC_000072.7:42222869-42227375	+
	Clcn1	NC_000072.7:42263552-42292698	+
	Zyx	NC_000072.7:42326491-42337147	+
	Epha1	NC_000072.7:42335421-42350249	-
	Arhgef5	NC_000072.7:43242578-43266254	+
	Nobox	NC_000072.7:43280608-43287006	-
Bos taurus	**TAS2R16**	NC_037331.1:87407411-87408316	-
	TAS2R62P	NC_037331.1:106839855-106840765	+
	TAS2R60	NC_037331.1:106849766-106850704	+
	TAS2R41	NC_037331.1:106880013-106880906	+
	AASS	NC_037331.1:86512542-86594714	-
	CADPS2	NC_037331.1:86734466-87314884	-
	SLC13A1	NC_037331.1:87464061-87568141	-
	GSTK1	NC_037331.1:106650764-106657919	+
	CLCN1	NC_037331.1:106700961-106737587	+

	ZYX	NC_037331.1:106775739-106788378	+
	EPHA1	NC_037331.1:106788864-106804343	-
	ARHGEF5	NC_037331.1:107620234-107651352	+
	NOBOX	NC_037331.1:107664889-107671402	-
Felis catus	***TAS2R16P***	NC_018724.3:142107645-142108538	-
	TAS2R62P	NC_018724.3:159082588-159083513	+
	TAS2R60P	NC_018724.3:159088520-159898445	+
	TAS2R41P	NC_018724.3:159113519-159114530	+
	AASS	NC_018724.3:141280711-141341987	-
	CADPS2	NC_018724.3:141473723-142022845	-
	SLC13A1	NC_018724.3:142159403-142300225	-
	GSTK1	NC_018724.3:158920615-158925017	+
	CLCN1	NC_018724.3:158963698-158995074	+
	ZYX	NC_018724.3:159030296-159039202	+
	EPHA1	NC_018724.3:159039378-159055821	-
	ARHGEF5	NC_018724.3:159676765-159700188	+
	NOBOX	NC_018724.3:159709505-159723783	-
Loxodonta africana	***TAS2R16AP***	NW_003573425.1:92807283-92808198	-
	TAS2R16BP	NW_003573425.1:92823476-92824492	-
	TAS2R16C	NW_003573425.1:92839824-92840705	-
	TAS2R62AP	NW_003573511.1:5351128-5351896	-
	TAS2R62B	NW_003573511.1:5408091-5409017	-
	TAS2R62C	NW_003573592.1:64061-65029	-
	TAS2R62D	NW_003573592.1:77090-78016	-
	TAS2R62EP	NW_003573592.1:92082-92942	-
	TAS2R62F	NW_003573592.1:109269-110249	-
	TAS2R60P	NW_003573592.1:46739-47643	-
	TAS2R41	NW_003573592.1:22267-23193	-
	AASS	NW_003573425.1:91897617-91946638	-
	CADPS2	NW_003573425.1:92073932-92686085	-
	SLC13A1	NW_003573425.1:92960950-93033713	-
	GSTK1	NW_003573592.1:304157-312773	-
	CLCN1	NW_003573592.1:221031-256464	-
	ZYX	NW_003573592.1:160502-163672	-
	EPHA1	NW_003573592.1:144292-160069	+
	ARHGEF5	NW_003573511.1:4886377-4916802	-
	NOBOX	NW_003573511.1:4869381-4873222	+
Echinops telfairi	***TAS2R16***	NW_022103920.1:22492917-22493816	+

	TAS2R60	NW_022103920.1:4180458-4181381	-
	TAS2R41	NW_022103920.1:4156860-4157786	-
	AASS	NW_022103920.1:23298673-23349431	+
	CADPS2	NW_022103920.1:22758009-23140073	+
	SLC13A1	NW_022103920.1:22239486-22339469	+
	GSTK1	NW_022103920.1:4331396-4340160	-
	CLCN1	NW_022103920.1:4283326-4305714	-
	ZYX	NW_022103920.1:4231912-4236636	-
	EPHA1	NW_022103920.1:4221368-4230998	+
	ARHGEF5	NW_022103920.1:3334725-3351326	-
	NOBOX	NW_022103920.1:3306873-3321536	+
Dasypus novemcinctus	*TAS2R16P*	NW_004474648.1:506090-506841	-
	TAS2R62P	NW_004487253.1:580420-581281	+
	TAS2R60P	NW_004487253.1:584749-585687	+
	TAS2R41	NW_004487253.1:612849-613775	+
	AASS	NW_004501360.1:576833-658124	+
	CADPS2*	NW_04474548.1:662323-897106	+
		NW_004501360.1:8138-371315	
	SLC13A1	NW_004474648.1:251754-357200	+
	GSTK1	NW_004487253.1:394086-399174	+
	CLCN1	NW_004487253.1:453990-492055	+
	ZYX	NW_004487253.1:523531-532663	+
	EPHA1	NW_004487253.1:532743-548607	-
	ARHGEF5	NW_004480844.1:479836-502679	-
	NOBOX	NW_004480844.1:450909-464185	-
Monodelphis domestica	*TAS2R60*	NC_008808.1:206393608-206394525	+
	TAS2R705	NC_008808.1:206525193-206526095	+
	TAS2R41	NC_008808.1:206551235-206552182	+
	AASS	NC_008808.1:179731018-179804769	-
	CADPS2	NC_008808.1:179991087-180731039	-
	SLC13A1	NC_008808.1:181009440-181114783	-
	GSTK1	NC_008808.1:20625076-206256965	+
	CLCN1	NC_008808.1:206340753-206377043	+
	ZYX	NC_008808.1:206464913-206474234	+
	EPHA1	NC_008808.1:206474453-206494286	-
	ARHGEF5	NC_008808.1:207268953-207329995	+
	NOBOX	NC_008808.1:207444632-207451867	-
Sarcophilus harrisii	*TAS2R60*	NW_003843673.1:192001-192912	+

	TAS2R705	NW_003843673.1:357999-358898	+
	TAS2R41	NW_003843673.1:339939-340883	+
	AASS	NW_003843660.1:2594381-2659468	-
	CADPS2	NW_003843661.1: 35110-586011	-
	SLC13A1	NW_003843662.1:15400-95809	-
	GSTK1	NW_003843673.1:84009-89226	+
	CLCN1	NW_003843673.1:138615-174265	+
	ZYX	NW_003843673.1:251173-259934	+
	EPHA1	NW_003843673.1:259714-279781	-
	ARHGEF5	NW_003843673.1:1054905-1082042	+
	NOBOX	NW_003843673.1:1270754-1276133	-
Phascolarctos cinereus	*TAS2R60*	NW_018344022.1:8316685-8317626	+
	TAS2R705A	NW_018344022.1:8540416-8541318	+
	TAS2R705B	NW_018344022.1:8581667-8582560	+
	TAS2R705C	NW_018344022.1:8819995-8820903	+
	TAS2R705DP	NW_018344022.1:8431864-8432867	-
	TAS2R705EP	NW_018344022.1:8490256-8491195	+
	TAS2R705FP	NW_018344022.1:8518197-8519267	-
	TAS2R705GP	NW_018344022.1:8528503-8529422	+
	TAS2R705HP	NW_018344022.1:8548204-8549169	+
	TAS2R705IP	NW_018344022.1:8569934-8570840	+
	TAS2R705JP	NW_018344022.1:8624230-8625169	+
	TAS2R705KP	NW_018344022.1:8653984-8654964	+
	TAS2R705LP	NW_018344022.1:8699145-8700106	+
	TAS2R705MP	NW_018344022.1:8740997-8741921	+
	TAS2R705NP	NW_018344022.1:8772735-8773698	+
	TAS2R41A	NW_018344022.1:8457114-8458043	+
	TAS2R41B	NW_018344022.1:8670223-8671242	+
	TAS2R41C	NW_018344022.1:8799763-8800710	+
	TAS2R41DP	NW_018344022.1:8591556-8592550	+
	TAS2R41EP	NW_018344022.1:8713039-8714104	+
	TAS2R41FP	NW_018344022.1:8849104-8850095	+
	TAS2R41GP	NW_018344022.1:8865547-8866552	+
	AASS	NW_018344118.1:3190171-3257650	-
	CADPS2	NW_018344118.1:3425919-4134012	-
	SLC13A1	NW_018344118.1:4369571-4480193	-
	GSTK1	NW_018344022.1:8169926-8184999	+
	CLCN1	NW_018344022.1:8262629-8298276	+

	ZYX	NW_018344022.1:8378140-8388076	+
	EPHA1	NW_018344022.1:8388307-8407479	-
	ARHGEF5	NW_018344022.1:9484469-9511895	+
	NOBOX	NW_018344022.1:9679198-9683747	-
Ornithorhynchus anatinus	**TAS2R810**	NC_041740.1:37671841-37672764	-
	TAS2R811	NC_041740.1:37668077-37669000	-
	TAS2R812	NC_041740.1:37663987-37664898	-
	TAS2R813A	NC_041740.1:37666009-37666935	-
	TAS2R813B	NC_041740.1:37661984-37662910	-
	TAS2R814P	NC_041740.1:37660283-37661126	-
	AASS	NC_041737.1:42596806-42640560	-
	CADPS2	NC_041737.1:42720031-43091041	-
	SLC13A1	NC_041737.1:43179512-43233468	-
	GSTK1	NC_041740.1:37544130-37547561	+
	CLCN1	NC_041740.1:37594471-37612012	+
	ZYX	NC_041740.1:37628065-37634099	+
	EPHA1	NC_041740.1:37634174-37645439	-
	ARHGEF5	NC_041740.1:37818542-37835225	+
	NOBOX	NC_041740.1:37835947-37839865	-
Tachyglossus aculeatus	**TAS2R810P**	NW_024044828.1:16466072-16467195	-
	TAS2R811P	NW_024044828.1:16463630-16464553	-
	TAS2R812AP	NW_024044828.1:16461219-16462129	-
	TAS2R812BP	NW_024044828.1:16457601-16458129	-
	TAS2R813	NW_024044828.1:16459202-16460131	-
	TAS2R814	NW_024044828.1:16455258-16456184	-
	AASS	NC_052075.1:39145291-39190568	-
	CADPS2	NC_052075.1:39276361-39647815	-
	SLC13A1	NC_052075.1:39749097-39838869	-
	GSTK1	NW_024044828.1:16322504-16326426	+
	CLCN1	NW_024044828.1:16379779-16397591	+
	ZYX	NW_024044828.1:16418956-16425027	+
	EPHA1	NW_024044828.1:16425139-16436641	-
	ARHGEF5	NW_024044828.1:16602677-16629480	+
	NOBOX	NW_024044828.1:16630100-16644680	-
Gallus gallus	**TAS2R1**	NC_006088.5:78481425-78482360	-
	TAS2R2	NC_006088.5:192365090-192366025	+
	TAS2R7	NC_006090.5:109851221-109852186	-
	AASS	NC_006088.5:23007392-23039846	+

CADPS2	NC_006088.5:22670262-22972029	+
SLC13A1	NC_006088.5:22565946-22594889	+
GSTK1	NC_006088.5:78425551-78436579	-
CLCN1	NC_006088.5:78261760-78334112	-
ZYX	NC_006088.5:78201043-78231480	-
EPHA1	NC_006088.5:78162527-78195974	+
ARHGEF5	NC_006088.5:74766817-74802789	-
NOBOX	NC_006088.5:74745799-74766293	-

CADPS2 of armadillo is separated into the two scaffolds. Its former part is in NW_004474648.1 and the latter is in NW_004501360.1.

Table S3 Compound library for the agonist screening assay

Compound	Vender[1]	Activated human TAS2Rs	N/S[2]	Tested conc. (mM)
Acesulfame K	WPCI	43, 31	S	10
Arbutin	Sigma-Aldrich	16	N	10
Caffeine	WPCI	7, 10, 14, 43, 46	N	1
Camphor	WPCI	4, 10, 14, 30	N	1
Chloramphenicol	WPCI	1, 8, 10, 39, 41, 43, 46	N	1
Colchicine	Sigma-Aldrich	4, 39, 46	N	10
Coumarin	Sigma-Aldrich	10, 14	N	0.3
Denatonium benzoate	WPCI	4, 8, 10, 13, 39, 43, 46, 30	S	3
Diphenidol	WPCI	1, 4, 7, 10, 13, 14, 16, 38, 39, 40, 43, 31, 46, 30, 20	S	0.1
Flufenamic acid	Sigma-Aldrich	14	S	0.03
Helicin	TCI	16, 43	N	10
Linamarin	SBC	16	N	20
Noscapine	Sigma-Aldrich	14	N	0.01
PTC	Sigma-Aldrich	38	S	0.1
Picrotoxin	WPCI	1, 10, 14, 46, 30 (Picrotoxinin)	N	1
Quinine HCl	Mylan	4, 7, 10, 14, 39, 40, 43, 31, 46	N	0.01
Saccharin	WPCI	8, 43, 31	S	10
Salicin	Sigma-Aldrich	16	N	40
Sinigrin	Extrasynthese	16, 38	N	1
Sodium benzoate	Nacalai Tesque, Inc.	14, 16	S	10
Sodium thiocyanate	WPCI	1, 38	S	3
Strychnine	Sigma-Aldrich	7, 10, 46	N	0.03
Thiamine HCl	WPCI	1, 39	N	1
Yohimbine	WPCI	1, 4, 10, 38, 46	N	0.3

[1]: WPCI: Wako Pure Chemical Industries, Ltd.; TCI: Tokyo Chemical Industry Co, Ltd.; SBC: Santa Cruz Biotechnology

[2]: N: natural compounds; S: synthetic compounds

Table S4 The amino acid residues, which affect the activation of human TAS2R16, in tested mammalian receptors

Domain	TM2				TM3						TM5			TM6			TM7				
BW numbering (GPCRdb)	53	57	60	61	32	33	35	36	39	40	39	43	48	51	52	55	39	42	43	45	46
Position in the multiple alignment	59	63	66	67	90	91	93	94	97	98	183	187	245	248	249	252	273	276	277	279	280
HumanTAS2R16	L	S	N	N	W	E	F	N	T	F	Q	H	F	Y	F	I	E	V	Y	F	I
CattleTAS2R16	Q	.	Y	.	.	.	T	.	S	.	S	K	.	.	.	V	.	I	.	L	V
ElephantTAS2R16C	R	.	Y	.	.	D	A	.	.	.	S	Q	.	.	.	L	.	I	.	V	S
OpossumTAS2R705	.	.	S	.	.	T	S	.	.	.	F	Q	Y	.	.	L	.	T	.	G	.
DevilTAS2R705	.	A	S	D	.	N	P	.	.	.	F	Q	.	.	L	L	.	T	.	G	.
QuollTAS2R705	.	A	S	D	.	N	P	.	.	.	F	Q	.	.	L	L	.	T	.	G	.
KoalaTAS2R705C	.	A	.	D	.	N	T	.	.	.	F	Q	C	.	V	L	.	T	.	S	L
WallabyTAS2R705B	.	A	S	.	.	S	T	.	.	.	L	Q	C	.	V	L	.	T	.	G	.
PlatypusTAS2R810	M	.	.	G	.	I	L	.	S	.	I	I	Y	.	.	L	Q	S	F	G	T
PlatypusTAS2R811	.	.	.	G	.	A	L	.	S	.	I	I	Y	.	.	L	Q	T	F	G	T
PlatypusTAS2R812	.	.	.	G	.	D	L	.	S	.	V	I	Y	.	.	L	Q	T	F	G	T
PlatypusTAS2R813A	.	A	.	G	G	V	L	G	S	.	I	T	Y	.	.	L	Q	T	.	G	P
PlatypusTAS2R813B	.	A	.	G	A	A	L	T	S	.	I	T	Y	.	.	L	Q	T	.	G	P
EchidnaTAS2R813	.	A	.	G	G	A	L	S	S	.	I	T	Y	.	.	L	Q	T	.	G	P
EchidnaTAS2R814	.	.	I	G	.	I	L	T	S	.	V	M	Y	H	.	L	Q	T	F	G	L
Sakurai et al., 2010						✓		✓		✓	✓	✓			✓	✓					
Thomas et al., 2017	✓				✓		✓	✓		✓			✓				✓	✓	✓		
Fierro et al., 2019	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Each coloration in amino acid positions shows how they affect TAS2R activation reported in Fierro et al., 2019. Orange and blue: ligand binding related to glucose moeity and aglycons, respectively; grey: ligand binding in specific ligands; white: formation of binding cavity. The check marks (✓) indicate the positions reported as the sites responsible for TAS2R16 activation in the paper.

Data set S1 Nucleotide sequences of TAS2Rs tested in the functional assays

>humanTAS2R16
ATGATACCCATCCAACTCACTGTCTTCTTCATGATCATCTATGTGCTTGAGTCCTTGACA
ATTATTGTGCAGAGCAGCCTAATTGTTGCAGTGCTGGGCAGAGAATGGCTGCAAGTCAG
AAGGCTGATGCCTGTGGACATGATTCTCATCAGCCTGGGCATCTCTCGCTTCTGTCTACA
GTGGGCATCAATGCTGAACAATTTTTGCTCCTATTTTAATTTGAATTATGTACTTTGCAA
CTTAACAATCACCTGGGAATTTTTTAATATCCTTACATTCTGGTTAAACAGCTTGCTTAC
CGTGTTCTACTGCATCAAGGTCTCTTCTTTCACCCATCACATCTTTCTCTGGCTGAGGTGG
AGAATTTTGAGGTTGTTTCCCTGGATATTACTGGGTTCTCTGATGATTACTTGTGTAACA
ATCATCCCTTCAGCTATTGGGAATTACATTCAAATTCAGTTACTCACCATGGAGCATCTA
CCAAGAAACAGCACTGTAACTGACAAACTTGAAAATTTTCATCAGTATCAGTTCCAGGC
TCATACAGTTGCATTGGTTATTCCTTTCATCCTGTTCCTGGCCTCCACCATCTTTCTCATG
GCATCACTGACCAAGCAGATACAACATCATAGCACTGGTCACTGCAATCCAAGCATGAA
AGCGCACTTCACTGCCCTGAGGTCCCTTGCCGTCTTATTTATTGTGTTTACCTCTTACTTT
CTAACCATACTCATCACCATTATAGGTACTCTATTTGATAAGAGATGTTGGTTATGGGTC
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